Field Dispersal and Survival of Sterile Medfly Males Aromatically Treated With Ginger Root Oil

BEATRIZ JORDÃO PARANHOS,[1,2] NIKOS T. PAPADOPOULOS,[3] DONALD MCINNIS,[4] CARLOS GAVA,[1] FABIANA S. C. LOPES,[1] RENATA MORELLI,[5] AND ALDO MALAVASI[6]

Environ. Entomol. 39(2): 570–575 (2010); DOI: 10.1603/EN08309

ABSTRACT We studied the dispersal behavior and survival of sterile medfly males either treated or not with ginger root oil (GRO), in field conditions, in Petrolina-PE, northeast Brazil, from May 2006 to December 2007 in a sterile insect technique (SIT) program. The *tsl* strain Vienna 8 from the *Ceratitis capitata* Wied. (Diptera: Tephritidae), medfly, mass-rearing facility located in Juazeiro-BA, Brazil, was used. The results showed that sterile males either exposed or not to GRO exhibit similar dispersal behavior and postrelease survival. More than 60% of the sterile males, either treated or not treared wth GRO, were recovered at a 25-m distance from the releasing point, ≈20% at 50 m, and 5% in traps situated 100 m from the releasing point. Around 90% of the sterile males, exposed or not to GRO, were recovered 5 d after release of the sterile male individuals, whereas <1% were recovered after 11 d. Our results imply that ginger root oil can be used to treat sterile medfly males without interfering with their dispersal or survival in the field.

KEY WORDS *Ceratitis capitata*, fruit fly, field dispersal, sterile insect technique

The sterile insect technique (SIT) is an environmentally friendly method to suppress insect pests and is widely used in worldwide programs against tephritid fruit flies, particularly the Mediterranean fruit fly (medfly), *Ceratitis capitata*, (Wiedemann) (Hendrichs et al. 2002). The success of the SIT depends vitally on the ability of released sterile males to disperse, find, and mate with wild females. Unfortunately, the mass-rearing process inherent to the SIT can often lead to a reduction in the quality of the released, sterile males compared with wild males (Shelly et al. 1994, Lance et al. 2000). Mass-rearing technology for the medfly has improved significantly to provide fruit fly factories around the world with sterile release populations containing only males to improve efficiency. In particular, temperature-sensitive lethal strains (*tsl*), in which female eggs are killed by the application of a high-temperature exposure period leaving only males surviving, have been developed and implemented worldwide (Robinson et al. 1999). Over generations of rearing in the laboratory, it is known that sterile males become less competitive at mating (Cayol 2000, Lance et al. 2000). Therefore, recent research efforts have focused on finding simple but effective means to improve the quality of sterile male fruit flies destined for field release in SIT programs worldwide. The performance of the sterile males in the wild is directly related with their ability to survive, disperse, and attract wild females, outcompete wild males, and finally mate with wild females.

It was relatively recently shown that exposure of *C. capitata* males to the odor of either orange oil or ginger root oil increases their mating performance (Papadopoulos et al. 2001, 2006; Shelly and McInnis 2001). The effect of the above compounds, especially that of ginger root oil, was subsequently tested, in a series of studies, with mass-reared sterile males. These studies clearly showed that the prerelease exposure of medfly males to the aroma of ginger root oil (GRO) (*Zingiber officinale* Roscoe), containing the known male attractant α-copaene significantly increases the mating competitiveness of sterile males (Shelly 2001, McInnis et al. 2002, Shelly et al. 2005, Paranhos et al. 2008). Exposures have increased in scale from small numbers of flies in small containers over a few hours to exposing millions of flies overnight in large rooms (Shelly et al. 2006). However, it remains unknown whether this aromatherapy treatment influences other important traits of sterile males such as their ability to disperse and survive in the field after being released. The objective of this work was to evaluate the dispersal behavior and field survival of sterile males treated or untreated with GRO before release in an SIT program.

[1] Laboratory of Fruit Flies, Brazilian Agricultural Research Corporation (Embrapa Tropical Semi Arid), BR 428, km 152, C.P. 23, 56.302-970 Petrolina-PE, Brazil.

[2] Corresponding author, e-mail: bjordao@cpatsa.embrapa.br.

[3] University of Tessaly, Department of Agriculture, Crop Production and Rural Environment, 38446 Volos, Greece.

[4] USDA–ARS Pacific Basin Agricultural Research Center, 2727 Woodlawn Dr., Honolulu, HI 96812.

[5] Department of Entomology, USP/ESALQ, C.P. 9, 13418-900 Piracicaba SP, Brazil.

[6] Medfly Facility of Brazil, 48.908-000 Juazeiro BA, Brazil.

0046-225X/10/0570–0575$04.00/0

Materials and Methods

Experiments were carried out in a commercial grape orchard, Timbaúba Farm (latitude: 09°13′ S, longitude: 40°29′ W), close to the Embrapa Laboratory Center, in Petrolina-PE, Brazil, from May 2006 to December 2007. The grape crop was in a vegetative phase after harvest or in the "rest phase" after pruning, with foliage to provide adequate shade for released flies.

Sterile males (*tsl* strain Vienna 8) from the medfly mass-rearing Moscamed Facility located in Juazeiro-BA, Brazil, were used in this experiment. Forty-eight to 24 h before emergence, pupae were dyed with fluorescent Day-Glo dye (300 ml of pupae with red color and 300 ml with blue color), packaged in plastic bags inside boxes with bags of iced gel, and sent by plane (1-h flight) to Federal University of Pernambuco, in Recife-PE. The next day, pupae received 95 Gy of gamma radiation from a Co^{60} source and were sent back to the Moscamed Facility in Juazeiro by plane. This entire procedure required ≈24 h, and the pupae temperature was checked soon after they arrived. Routine quality control (QC) tests were carried out with the two colors of pupae (red and blue) to verify pupal weight, number of pupae per milliliter, percentage of emergence, and percentage of fliers. After the QC tests were completed, six plastic screened cages (3 liters) per color were set up with 40 ml of pupae each. All cages were provided with honey jelly (agar + water + honey) and water and kept in a room with controlled temperature and humidity (25 ± 2°C; 55 ± 10% RH). On day 4 or 5 after emergence, one of these sets of flies, red or blue, was exposed to 1.5 ml of GRO in a small room (27 m^3) for 20 h, 25–27°C, and 50–60% RH, with a 12-h photoperiod, in which the GRO aroma was dispersed by air fans.

At the end of GRO treatment, around 0800 hours, flies from 12 cages, 6 exposed and 6 not exposed to GRO, were released in the center of the 25-ha grape orchard. For each dispersal test, ≈20,000 males, 10,000 exposed and 10,000 not exposed to GRO, marked with red and blue fluorescent dye, alternately, were released. To monitor male dispersion and survival, a grid of 48 Jackson traps baited with a trimedlure plug (t-butyl-2-methyl-4-chlorocyclohexanecarboxylate; Bio Ceratitis) were placed in circles at 25, 50, 100, 150, 200, and 250 m from the release point in eight directions: north, south, east, west, northeast, northwest, southeast, and southwest. Preliminary studies showed that most sterile males were recovered within 50 m from the releasing point; however, we included in our experiments traps at longer distances to determine longest dispersion of the released flies. The trap positions were determined by GPS. Traps were exposed in the field 1, 3, 5, 7, 9, and 11 d after release for 2, 4, 6, 8, 24, and 24 h, respectively. Captured flies at each trap position were brought to the laboratory for scoring. Sterile Vienna-8 males (red or blue) were separated from wild males under a UV light source based on the presence or absence of the different dye colors. Five replicates of the complete experiment were carried out. Climatic data (temperature, wind speed, solar irradiation, and rainfall) were collected daily from a meteorological station 2 km from the experimental area.

The experiment was factorial, with four factors: type of male (2 = treated and untreated with GRO), distance (6), time after release (6), and direction (8). Data collected were numbers of sterile males, treated and untreated with GRO, recovered from traps. Data were submitted to analysis of variance (ANOVA; Statistica for Windows v 5.5) and Tukey's test to examine differences among treatments and averages, respectively. In cases where there were no significant differences between treated and untreated males, we used sums of the two types of sterile males to test effects of distance direction and postrelease time. The experiment was conducted in a randomized block design. To determine the ability of sterile males, exposed and not exposed to GRO, to disperse in an area (S^2) and in linear meters (DM), the following model of Dobzhansky and Wright (1943) was adopted:

where S^2 (m^2) = variance of dispersal area (m^2) during the experimental period; DM = median distance = middle distance moved of flies trapped during the experimental period; r = distance (m) of traps from the center; a = number of traps in each circle ($n = 8$ for all circles); i = total number of sterile males recovered in each circle (consider each distance a circle's radius); and c = average number of sterile flies trapped in the first circle (with $r = 25$ m) for each trapping day (eight traps) over the five replications combined.

Results

Although we did not perform any correlation analyses between the number of flies recovered and the climatic data, the low variation of the climatic conditions during the different experimental trials (Table 1) indicated no differential effects of the above conditions on dispersion and survival of the released males. Temperatures (median, minimum, and maximum) and solar radiation were slightly lower during May and June compared with October and November, and the relative humidity was a little higher.

There was no significant difference between the total numbers of the two groups of sterile males (exposed or not exposed to GRO) recovered in the traps [$F(1,2747) = 1.98$; $P = 0.159$; $N = 2,872$]. The total numbers of males capture were 3,357 for those exposed to GRO and 4,168 for those not exposed. These values correspond to 6.71 and 8.34% of the total number of released males for those exposed and not exposed to GRO, respectively.

There were no significant differences in the number of flies recovered at each distance between the two types of males [$F(5,2747) = 0.599$; $P = 0.700$; $N = 2,872$]. However, regardless of the type of males (GRO exposed or not exposed), there was a significant effect of the distance from the releasing point on the total number of recovered sterile males [$F(5,2747) =$

Table 1. Average maximum, median, and minimum temperatures, average relative humidity, average total solar radiation (SR) per day (MJ/m^2), average wind speed (WS), total rainfall obtained during five experimental periods, and percentage of flies (treated = GRO+ and untreated = GRO−) recovered in each release

Experimental period	Temp (med) (°C)	Temp (max) (°C)	Temp (min.) (°C)	Relative humidity (med) (%)	Relative humidity (max) (%)	Relative humidity (min.) (%)	SR (MJ/m^2)	WS (m/s)	Rain (mm)	Flies GRO+ (%)	Flies GRO− (%)
31 Oct. to 10 Nov. 2006	27.43	34.18	21.15	57.39	84.09	32.77	24.71	2.27	0.00	5.12	14.57
22 Nov. to 3 Dec. 2006	26.51	32.95	21.12	64.16	85.59	38.26	24.94	2.09	10.22	12.5	3.77
16–27 May 2007	24.84	31.36	19.73	69.81	92.06	41.68	18.97	2.47	6.86	4.65	8.17
5–16 June 2007	23.99	30.66	18.05	65.80	91.04	39.15	18.06	2.58	0.25	3.98	5.36
5–16 March 2008	26.56	33.02	21.82	67.37	88.44	37.7	25.04	1.38	14.98	7.63	9.87

203.87; $P \leq 0.0001$; $N = 2{,}872$]. All two-way interactions among factors (type of male, distance, time after release, and direction) were not significant ($P > 0.05$). Approximately 90% of the recovered flies, both for GRO-treated and nontreated flies, were recovered up to 100 m from the release point, and only 10% of flies were detected at a distance >150 m from the release point. For both GRO-treated and nontreated males, there were significantly more flies recovered at traps placed 25 m from the releasing point compared with traps placed at longer distances. Likewise, significantly more flies were recovered at 50 m from the release point compared with longer distances (Fig. 1). Traps placed at a distance >50 m from the release point captured similar number of males (Fig. 1).

The best-fitting curves for exponential regression in relationship to the flight distances dispersed for exposed and not exposed males are shown in Fig. 2. The two curves almost overlap each other; therefore, both types of sterile males exibited very similar dispersion behavior.

We did not find a significant difference between sterile males, exposed and not exposed to GRO, recovered on each of the experiment days [$F(5,2747) = 1.558$; $P = 0.168$; $N = 240$]. However, postrelease time (days after release) significantly affected recovery rates of both groups of sterile males [$F(5,2747) = 23.37$; $P \leq 0.0000$; $N = 2{,}872$]. Approximately 90% of sterile males, exposed and not exposed to GRO, were recovered up to day 5 after release, whereas only 10% of sterile males were recovered from day 7 onward after release (Fig. 3). The average number of both groups of sterile males trapped on the first day was significantly higher than that recovered on the third day, and on the third day it was significantly higher than 5, 7, 9, and 11 d after release (Tukey test, $P \leq 0.05$; Fig. 4).

The direction of the traps did not affect the number of males recovered for both groups of males (GRO exposed and not exposed) [$F(7, 2747) = 0.109$; $P = 0.99$, and $F(7,2747) = 1.162$; $P = 0.321$, for GRO exposed and not exposed males, respectively].

As shown in Fig. 5, the postrelease time (days after release) similarly affected both dispersion distance and dispersion area of the GRO exposed and nonexposed males. The longest distance and widest area were recorded for both males on day 9 after release.

Discussion

The percentage of recovered sterile males was similar for both types of males (treated and nontreated).

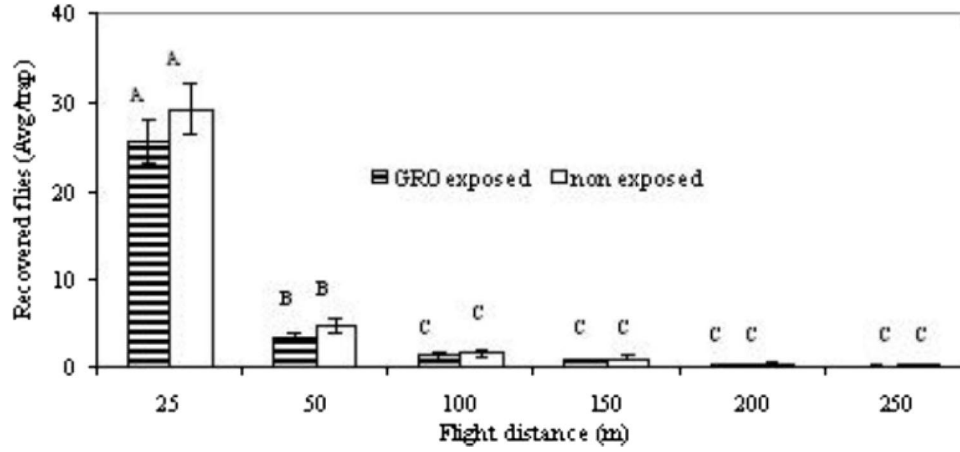

Fig. 1. Average numbers (±SE) of sterile males exposed and not exposed to GRO recovered at 25, 50, 100, 150, 200, and 250 m from the release point in a grape orchard. Bars with the same letter from the same treatment do not differ significantly (Tukey's test, $P < 0.05$). There was not difference between averages of exposed and not exposed recovered males in each distance.

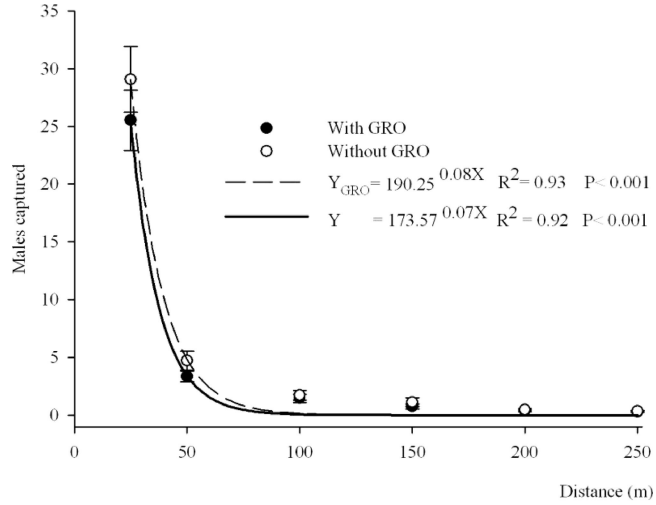

Fig. 2. Regression curve with equations between flight range (m) and numbers of sterile males trapped, exposed and not exposed to GRO, from a grape orchard in northeastern Brazil.

These findings suggest similar dispersion and survival abilities of GRO-exposed and non-exposed flies, based on similar responses to traps. Shelly et al. (2006) found significantly more GRO-exposed males captured in traps than nonexposed ones. However, Shelly et al. performed aerial releases of sterile flies over large areas where the trapping data do not allow for a direct comparison of the dispersal ability of exposed and nonexposed males. The recovery rates of both groups of males found here are in agreement with those reported by Wong et al. (1982) in Hawaii. However, Paranhos et al. (2006) observed a higher recovery rate (16.20%) in a mango orchard in Brazil.

Our results showed a similar flight range between GRO-exposed and nonexposed males and similar temporal and spatial dispersion behavior. Similar effects have been reported earlier by Shelly et al. (2006) for *tsl*-sterile males released by aircraft in Florida. In another study, Shelly et al. (2007) did not find a statistical difference for recovered males, exposed and not exposed to GRO, when released at several points in a coffee orchard.

Around 90% of sterile males, exposed and not exposed to GRO, caught in the 48 Jackson traps in the grape orchard, were recovered within 5 d after release (consistent with Baker and Chan 1991, Vargas et al. 1995, Paranhos et al. 2006) and up to 100 m from the release point (consistent with Wong et al. 1982, Vargas et al. 1995, Paranhos et al. 2006). The frequency of recovery was much higher (60%) on the first day and at 25 m from the release point. This might be related to a tendency of released males to remain close to the release point, their inability to disperse longer distances, and their field survival. Our results suggest that dispersion of flies out of the experimental trapping grid, if any, would be negligible. Flies that manage to

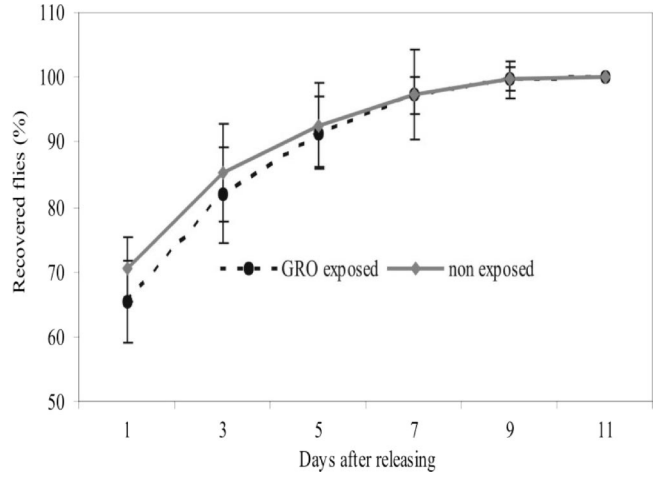

Fig. 3. Average numbers (±SE) of cumulative percentage of sterile males exposed ($n = 5$) and not exposed to GRO ($n = 5$), recovered 1, 2, 3, 5, 7, 9, and 11 d after release in a grape orchard.

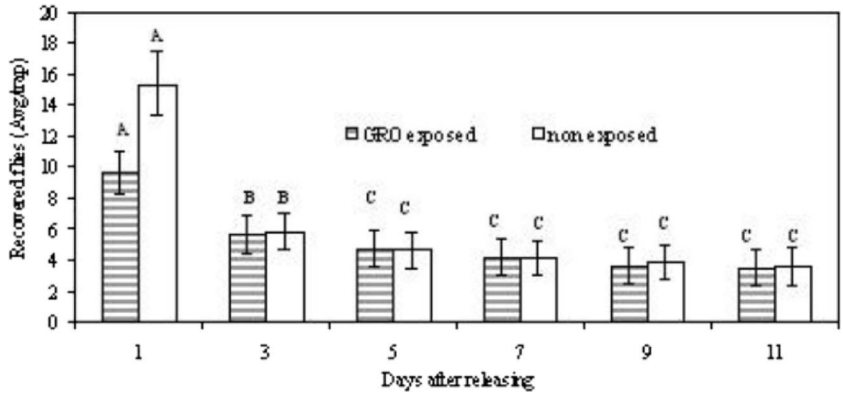

Fig. 4. Average number of sterile males (±SE), exposed and not exposed to GRO recovered per trap 1, 3, 5, 7, 9, and 11 d after release in a grape orchard. Bars with the same letter belonging to same treatment do not differ significantly (Tukey's test, $P < 0.05$). There was not difference between averages of exposed and not exposed recovered males, in each capture day.

survive >2 d after release might have continued dispersing, reaching greater distances and spreading out over larger areas. In the case of an irrigated grape orchard in the San Francisco River Valley, the grapevines provided a continuous covering at ≈1.9 m above ground. Thus, the conditions in the area (shade, food, water, temperature) were quite uniform. Despite no flowers or ripe fruits for foraging, the sterile males did not disperse much from the release point and did not survive for many days because of scarce food. Wong et al. (1982) also found a low percentage of recovered sterile males released in an orchard lacking ripe fruits.

Maor et al. (2004) found that protein-fed *tsl*-sterile males died within 2 d after released in an environment without food but with water available. The authors showed that protein-deprived males survived well under starvation conditions after release, whereas protein-fed males do not. Previous studies carried out in our laboratory showed that a protein-supplemented diet did not increase sterile male survival (unpublished data). Because it is expected that sterile males will only survive up to a few days in the field after release, it will be important for the flies to be released after they reach sexual maturity (4–5 d old). If they are ready to mate when released, surviving 2–3 d and being released twice a week would be enough to accomplish their role in a successful SIT program.

GRO aromatherapy did not modify the dispersal behavior or survival of sterile males, *tsl* Vienna 8 strain, of *C. capitata* in the field. The dispersal behavior of

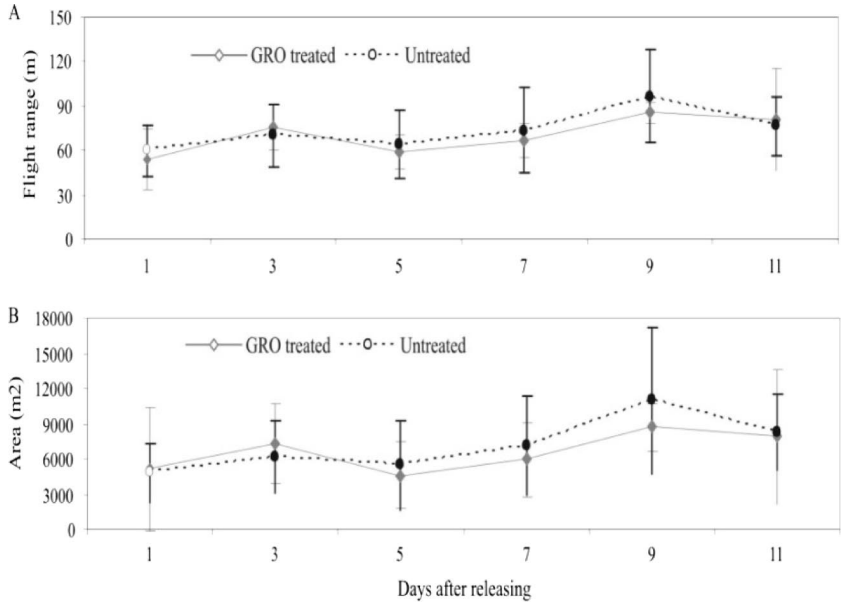

Fig. 5. Average numbers (±SE) of flight distance ($n = 5$) (A) and area covered ($n = 5$) (B) by sterile males, exposed and not exposed to GRO, after 1, 3, 5, 7, 9, and 11 d after release in a grape orchard.

sterile males, *tsl* Vienna 8 strain, of *C. capitata* was satisfactory in a commercial grape orchard located in the semiarid region of northeast Brazil. GRO aromatherapy did not modify their dispersal behavior or survival in the field. Therefore, GRO can be used on an industrial scale to improve the sexual performance of sterile medfly males and enhance the efficacy of SIT program.

Acknowledgments

We thank Moscamed Facility Brazil for supplying irradiated pupae for these studies; V. Borges, D. Alves, G. dos Santos, and G. de Souza for collecting traps in the field; and M. de Sá, R. de Castro, I. Souza, and M. Fabrício de Barros for helping us with the experiments.

References Cited

Baker, P. S., and A.S.T. Chan. 1991. Quantification of tephritid fruit fly dispersal. Guidelines for a sterile release programme. J. Appl. Entomol. 112: 410–421.

Cayol, J. P. 2000. Changes in sexual behavior and in some life history traits of tephritid species caused by mass-rearing processes, pp. 843–860. *In* M. Aluja and A. Norrbom (eds.), Fruit flies (Tephritidae): phylogeny and evolution of behavior. CRC, Boca Raton, FL.

Dobzhansky, T., and S. Wright. 1943. Genetics of natural populations X dispersion rates in *Drosophila pseudoobscura.* Genetics 28: 304–340.

Hendrichs, J., A. S. Robinson, J. P. Cayol, and W. Erkenlin. 2002. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behavior studies. Fla. Entomol. 85: 1–13.

Lance, D. R., D. O. McInnis, P. Rendon, and C. G. Jackson. 2000. Courtship among sterile and wild *Ceratitis capitata* (Diptera: Tephritidae) in field cages in Hawaii and Guatemala. Ann. Entomol. Soc. Am. 93: 1179–1185.

Maor, M., B. Kamensky, S. Shloush, and B. Yuval. 2004. Effects of post-teneral nutritional on foraging success os sterile male Mediterranean fruit flies. Entomol. Exp. Appl. 110: 225–230.

McInnis, D. O., T. E. Shelly, and J. Komatsu. 2002. Improving male mating competitiveness and survival in the field for medfly, *Ceratitis capitata* (Diptera: Tephritidae) SIT programs. Genetics 116: 117–124.

Papadopoulos, N. T., B. I. Katsoyannos, and N. A. Kouloussis. 2001. Effect of orange peel substances on mating competitiveness of male *Ceratitis capitata.* Entomol. Exp. Appl. 99: 253–261.

Papadopoulos, N. T., T. E. Shelly, N. Niyazi, and E. Jang. 2006. Olfactory and behavioral mechanisms underlying enhanced mating competitiveness following exposure to ginger root oil and orange oil in males of the mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). J. Insect Behav. 19: 403–418.

Paranhos, B. J., D. O. McInnis, K. Uramoto, I. Damasceno, N. Gonçalves, R. M. Alves, M.L.Z. Costa, J. Walder, A. Malavasi, and A. Nascimento. 2008. Sterile medfly males of the tsl Vienna 8 genetic sexing strain improved mating performance with ginger root oil, pp. 313–318. *In* R. L. Sugayama, R. A. Zuchi, S. M. Ovruski, and J. Sivinski (eds.), Fruit Flies of Economic Importance: From Basic to Applied Knowledge.

Paranhos, B. J., N. T. Papadopoulos, I. Damasceno, M. Romano, R. Alves, R. Lopes, M.L.Z. Costa, A. Malavasi, A. S. Nascimento, and J.M.M. Walder. 2006. Dispersão e sobrevivência de machos estéreis de moscamed, linhagem mutante *tsl*, no submédio do Vale do São Francisco. *In* Congresso Brasileiro de Entomologia, 21, 2006, Recife. Resumos. Recife: SEB: UFRPE 2006. 1 (CD-ROM).

Robinson, A. S., G. Franz, and K. Fisher. 1999. Genetic sexing strains in the medfly, *Ceratitis capitata:* development, mass-rearing, and field application. Trends Entomol. 2: 81–104.

Shelly, T. E. 2001. Exposure to alpha-copaene and alpha-copaene-containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae) Ann. Entomol. Soc. Am. 94: 497–502.

Shelly, T. E., and D. O. McInnis. 2001. Exposure to ginger root oil enhances the mate competitiveness of irradiated, mass reared males of the Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 94: 1413–1418.

Shelly, T. E., T. S. Whittier, and K. Kaneshiro. 1994. Sterile insect release and the natural mating system of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 87: 470–481.

Shelly, T. E., D. O. McInnis, and P. Rendon. 2005. The sterile insect technique and the Mediterranean fruit fly: assessing the utility of aromatherapy in large field enclosures. Entomol. Exp. Appl. 116: 199–208.

Shelly, T. E., T. C. Holler, and J. L. Stewart. 2006. Mating competitiveness of mass-reared males of the Mediterranean fruit fly (Diptera: Tephritidae) from eclosion towers. Fla. Entomol. 89: 380–387.

Shelly, T. E., D. O. McInnis, C. Rodd, J. Edu, and E. Pahio. 2007. Sterile insect technique and Mediterranean fruit fly: assessing the utility of aromatherapy in a Hawaiian coffee field. J. Econ. Entomol. 100: 273–282.

Vargas, R.I.L. Whitehand, W. A. Walsh, J. P. Spencer, and C. L. Hsu. 1995. 1995. Aerial releases of sterile Mediterranean fruit fly (Diptera: Tephritidae) by helicopter: dispersal, recovery, and population suppression. J. Econ. Entomol. 88: 1279–1287.

Wong, T.T.Y., L. C. Whitehand, K. O. Kobayashi, N. Tanaka, and E. Harris. 1982. Mediterranean fruit fly: dispersal of wild and irradiated and untreated laboratory-reared males. Environ. Entomol. 11: 339–343.

Received 19 December 2008; accepted 5 November 2009.